

# Vascular Endothelial Growth Factor in Aqueous Humor of Patients with Perforative Eye Injuries

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## ABSTRACT

*Considering that VEGF is the key factor for angiogenesis stimulation, we wanted to establish if VEGF level is increased in aqueous humor of patients with open globe eye injury. The study included 20 patients with open globe injury. During the surgery, aqueous humor samples were taken out and VEGF levels were measured by ELISA. VEGF levels were significantly higher in the aqueous humor of patients with open globe eye injury and uveitis, in patients with wound bigger than 2 mm and in patients where from injury to surgery passed more than 4 hours. VEGF levels were also higher, but not significantly, in patients with intrabulbar foreign body. Considering that VEGF levels were significantly higher in patients with open globe eye injury with uveitis, wound larger than 2 mm and in patients where from injury to surgery passed more than 4 hours, anti VEGF therapy might have application in these conditions.*

**Key words:** vascular endothelial growth factor, aqueous humor, perforative eye injury

## Introduction

Eye injuries are rather common and are frequent cause of visual damage. Mechanical injuries, burns and electrocutions take the first spot. Superficial eye injuries are most frequent and only 5% is made up of perforating injuries affecting deeper eye structures. Injured patients are mostly between 15 and 60 years of age and 80% of them are men.

Corneal injury treatment aims at reestablishing the eye's integrity and leaving the least possible amount of scars which could impair vision. With the aim of preventing infection and consequent inflammation, and leaving as little scars as possible, antibiotics are administered locally and systematically, sometimes even corticosteroids. New treatment methods are based on blocking the influence of the vascular endothelial growth factor (VEGF).

The data published by researchers in the field and the results achieved until now point towards the fact that vascularisation which follows scarring actually is one of the main causes of complications arising after a perforating injury. Increased VEGF values have been recorded in perforating injuries and vascular corneal leukomas, oc-

curing as a consequence, as well as illnesses pertaining to the posterior eye segment, when neovascular membranes are created.

Trauma is the leading cause of blindness in children and young adults, as well as a significant cause of blindness in older individuals. About 75% of people with trauma-induced visual impairment are monocularly blind. In the last 50 years, significant advances in the treatment of ocular trauma have been achieved. The development of microsurgical techniques, the use of an operating microscope and the development of vitrectomy today enable visual acuity recovery of an eye which would have previously been enucleated.

Almost 50% of all eye injuries take place in children who are less than 18 years of age<sup>1</sup>. Open eye injuries in children cause great difficulties in the evaluation of complications and monitoring during the postoperative period<sup>2-7</sup>.

Perforating injuries can be classified according to the mechanisms which caused them, into sharp, blunt, and

projectile-induced. Injuries can also be classified on the basis of whether an intraocular foreign body (IOFB) is present or not, the type of surgical intervention needed, as well as the length of postoperative treatment. Patients are monitored according to their sex, age, affected eye, best-corrected visual acuity, afferent pupillary defect, localization and size of the wound, hyphemas, presence of cataract, iris and vitreous body prolaps, vitreous hemorrhage, retinal ablation, intraocular foreign body (IOFB), required vitrectomy and type of injury. All the aforementioned factors are evaluated as predisposing factors for final visual acuity, possible retinal ablation and a possible need for eyeball enucleation<sup>1,8,9</sup>.

Considering that vascular endothelial growth factor is the key factor for angiogenesis stimulation, we wanted to establish if VEGF level increased in aqueous humor of the patients with open globe eye injury and how it depends on the injury size, presence of inflammation, incoming vision, the extent of the injury affected area, time passed from the injury occurrence to its treatment and existence or absence of intrabulbar foreign body.

## Patients and Methods

The study included 20 patients with open globe eye injury; 16 male (80%), 4 female (20%); from the Department of Ophthalmology, University Hospital Rijeka from January 2006 to January 2008. During the surgery in patients with open globe eye injury the aqueous humor samples were taken (needle Medoject 26G, syringe Chirana 2 ccm) and VEGF levels were assayed by ELISA (R&D System, USA). Controls consisted of the sample of aqueous humor taken from cadavers. The data was collected and analyzed by STATISTICA 7,1 classic descriptive method.

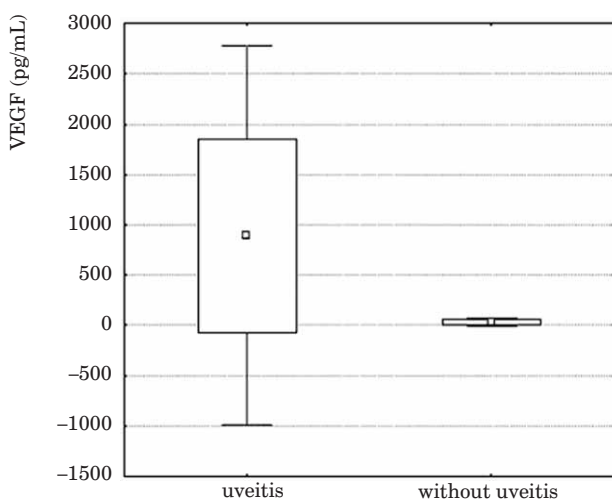


Fig. 1. VEGF levels in aqueous humor in patients with perforating eye injury with uveitis and without uveitis.

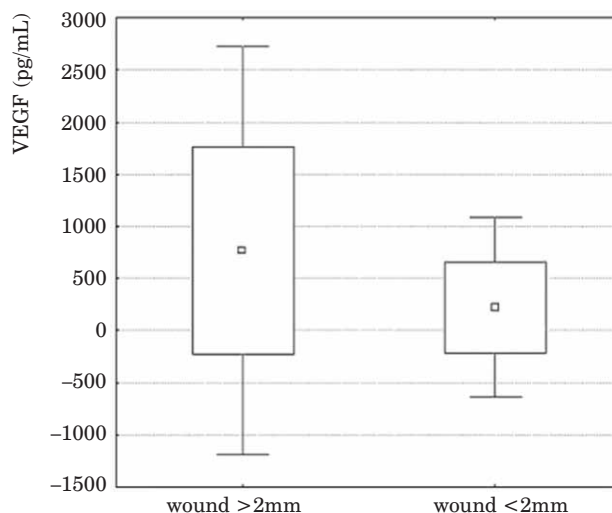


Fig. 2. VEGF levels in aqueous humor in patients with perforating eye injury with wound bigger than 2 mm and with wound less than 2 mm.

## Results

In the aqueous humor VEGF levels were significantly higher in patients with open globe eye injury and uveitis (887.77 pg/mL) than in patients without uveitis (27.92 pg/mL,  $p < 0.005$ ) (Figure 1).

Aqueous humor VEGF levels were also higher, but not significantly, in patients with open globe eye injury and wound bigger than 2 mm (760.06 pg/mL) than in patients with open globe eye injury and wound less than 2 mm (219.48 pg/mL) (Figure 2).

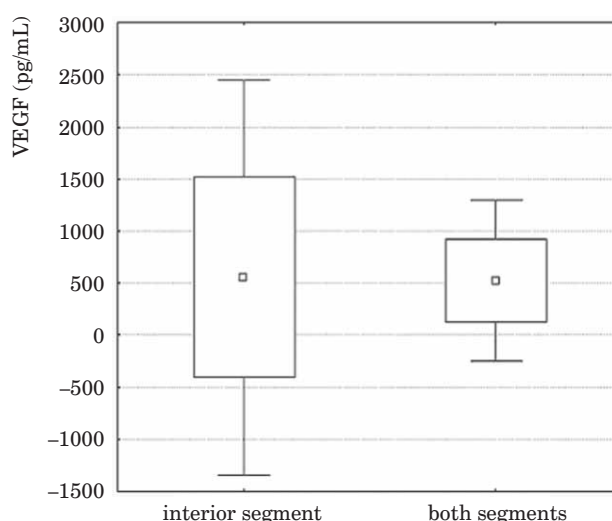


Fig. 3. VEGF levels in aqueous humor in patients with perforating eye injury and injured anterior segment and patients with injured both segments.

In the aqueous humor of patients with open globe eye injury and only anterior segment damage, VEGF quantity was not significantly higher (518.52 pg/mL) than in patients with both segments injured (552.13 pg/mL) (Figure 3).

In the aqueous humor of patients with open globe eye injury and incoming vision less than 0.1, VEGF quantity was not significantly higher (549.96 pg/mL) than in patients with incoming vision more than 0.1 (525.44 pg/mL) (Figure 4).

In the aqueous humor of patients with open globe injury, where from injury to surgery passed more than 4 hours, VEGF levels were significantly higher (948.27 pg/mL) than in patients where from injury to surgery passed less than 4 hours (212.92 pg/mL,  $p < 0.005$ ) (Figure 5).

In the aqueous humor VEGF levels were higher, but not significantly, in patients with open globe eye injury and intrabulbar foreign body (671.26 pg/mL) than in patients with open globe eye injury without intrabulbar foreign body (161.52 pg/mL) (Figure 6).

## Discussion and Conclusion

Literature data reports that almost 50% of all eye perforation injuries occur with the children less than 18 years of age. Taking into consideration the common bad sight rehabilitation in those conditions, it is essential to examine the factors aggravating the sight prospects of a perforated eye. Vascularization and connective tissue proliferation are the key factors generating post injury complications and their prospects are quite unpromising at all since lesion cicatrices and neovascularization lead to blindness<sup>1</sup>.

Considering that vascular endothelial growth factor is the key factor for angiogenesis stimulation, we wanted to

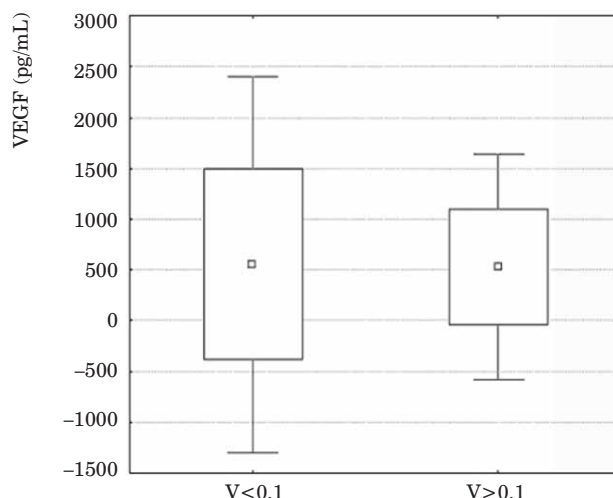


Fig. 4. VEGF levels in aqueous humor in patients with perforating eye injury and incoming vision less than 0.1 and injured anterior segment and patients with incoming vision more than 0.1.

establish if VEGF level increase in aqueous humor of the patients with open globe eye injury and how it depends on the injury size, presence of inflammation, incoming vision, the extent of the injury affected area, time passed from the injury occurrence to its treatment and existence or absence of intrabulbar foreign body.

In the tested group, 60% of patients have shown marked inflammation signs under slit-lamp exam. We proceeded to compare their VEGF rate level with the VEGF rate level in patients showing no such inflammation signs. The research results have revealed that VEGF levels were significantly higher in the patients with marked inflammation signs (888.77 pg/mL) in relation to

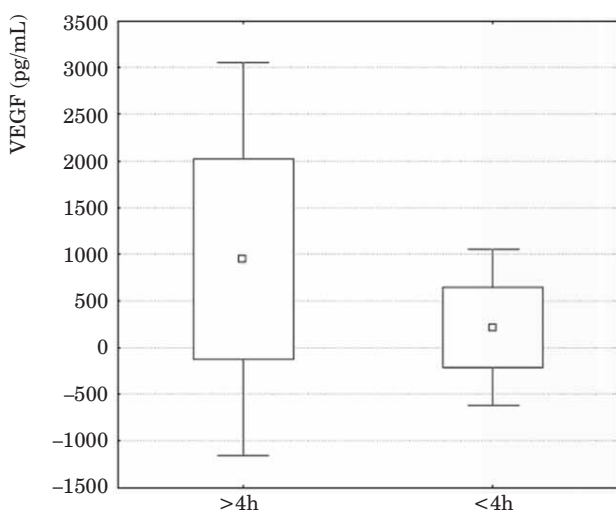


Fig. 5. VEGF levels in aqueous humor in patients with perforating eye injury where from injury to surgery passed more than 4 hours and in patients where from injury to surgery passed less than 4 hours.

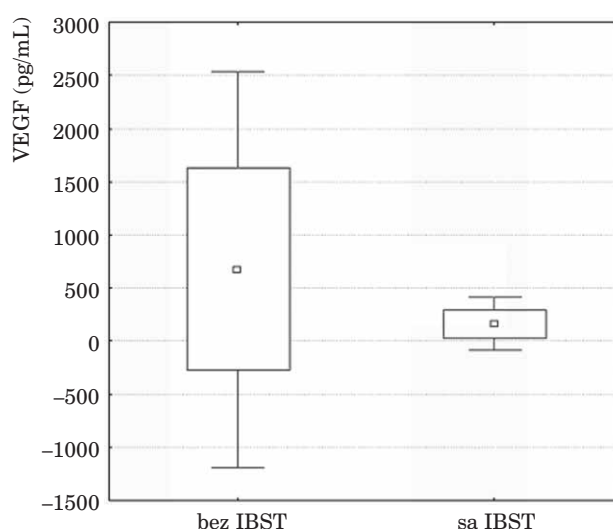


Fig. 6. VEGF levels in aqueous humor in patients with perforating eye injury and intrabulbar foreign body and in patients with open globe eye injury without intrabulbar foreign body.

the patients with no such inflammation signs (27.92 pg/mL) which demonstrates that those patients are more susceptible to exert possible pathologic vascularization and thereby poorer sight rehabilitation.

There is no literature data available regarding to VEGF rate level in the perforative eye injuries, but on the other hand, there are the VEGF rate levels under inflammation conditions which show clinical similarity to a perforated eye. Howard et al have reported that a considerably higher VEGF rate level has been measured in the aqueous humor (152.3 pg/mL and 109.5 pg/mL) and plasma in patients with uveitis and cystoid macular edema than in those only affected by signs of uveitis<sup>10,11</sup>.

The mean VEGF levels in patients belonging to the control group went from 29.6 pg/mL to 55.0 pg/mL. Considering the fact that our patients with open globe eye injury also showed clinical signs of uveitis (injury induced), our results correlate closely with this study. VEGF increase in patients with perforating injuries, which demonstrated more visible signs of inflammation, indicates that VEGF is one of the inflammatory mediators (probably alongside other factors as well) responsible for inducing uveitis in the affected eyes.

In our study we have also evaluated whether wound size influences increased VEGF production (patients were divided up between those having a wound which was either larger or smaller than 2 mm). Twelve patients had wounds which were larger than 2 mm and the results of their VEGF quantity measurements showed that the increased VEGF quantities were not statistically significant (760.06 pg/mL), if compared to patients whose wounds were smaller than 2 mm (219.48 pg/mL). The above indicates that perforating wound length does not necessarily have a direct influence on the initiation of an immunological reaction and the production of vasogenic factors.

Our research was based on the assumption that final visual acuity could also depend on the production of pro-inflammatory cytokines, such as VEGF, and we concentrated on the differences in VEGF production, depending on whether the injury had occurred in only one or both eye segments. The analysis of the results showed that there is no statistically significant difference between the aforementioned two groups, as regards the quantity of VEGF deriving from the measurements (552.13 pg/mL and 518.92 pg/mL). The above can be explained by the fact that, once it is stimulated, the immunological reaction has a potential for subsequent spreading, also to the eye segment which was not directly struck by the injury. This fact can also be corroborated by clinical experience, as anterior eye segment perforations have a cause-effect relationship with cystoid macular edema (an illness pertaining to the posterior eye segment).

Initial visual acuity is usually considered to be a predictor of final visual acuity. Stenberg et al. found that initial visual acuity of 20/800 or greater is one of the strongest predictors of final visual acuity. Esmaeli et al. detected visual acuity of HM or less, wound length greater than 10 mm and injury types for which vitrectomy

was needed, as negative factors for final visual acuity. In our study 13 patients had a visual acuity of less than 0.1. The analysis of the VEGF quantity measurement results showed that there is no statistically significant difference between the quantity of the VEGF deriving from the measurements performed on patients with an initial visual acuity of less than 0.1 (549.96 pg/mL) and those with an initial visual acuity which was greater than 0.1 (525.44 pg/mL). Clearly immunological reactions are merely one of the factors which can influence final visual acuity. All the above confirms the fact that visual acuity measurements at the time of the injury cannot be used as a determining factor for visual prognosis because eye perforation is a very complicated condition, influencing visual acuity through numerous factors.

Twenty-five percent of the patients who participated in our study presented a perforating injury with an intrabulbar foreign body present. The analysis of the VEGF quantity measurement results showed that the quantity of VEGF in patients having an intrabulbar foreign body (671.26 pg/mL) is greater than the quantity of VEGF measured in patients with no intrabulbar foreign body (161.52 pg/mL), however the difference is not statistically significant. Notwithstanding the lack of a statistically significant difference, clinical experience tells us that certain foreign bodies stimulate neovascularisation in their environment. The intrabulbar foreign bodies present in our research were made out of inert materials (metal and glass), which explains the statistical insignificance as regards the different quantities of VEGF between patients with an intrabulbar foreign body and without it.

In 45% of the cases covered by our study more than 4 hours had passed from the moment when the injury actually occurred to when the wound was treated, and the remaining 55% of patients were taken care of and treated in the operation room within the first 4 hours from when the injury had occurred. The analysis of the VEGF quantity measurement results showed a greater quantity of VEGF (representing a statistically significant difference) in patients that had been treated after more than 4 hours had passed from the moment of the injury (948.27 pg/mL), if compared to VEGF quantities in patients whose wounds were treated within 4 hours from when the injury took place (212.92 pg/mL). The assumption is that the more time passes from the moment in which an injury actually occurs to the treatment of the wound, the more signs of inflammation will develop, which is why the production of greater VEGF and interleukine-8 quantities can be expected, explaining the statistically significant difference between the observed groups. It is namely a fact, as mentioned above, that VEGF and interleukine-8 are angiogenic mediators demonstrating a potent pro-inflammatory activity<sup>12</sup>, which connects them to the occurrence of uveitis and proliferative vitreo-retinopathy, jeopardizing the visual outcome of the injured eye.

Finally, recent studies have mentioned anti VEGF therapy in the treatment of cystoid macular edema<sup>11</sup>. This is why there is a theoretical possibility of applying

such a therapy also to the prevention of cystoid macular edema after eye perforation. The results of research performed on perforating eye injury patients have for the first time suggested the advisability of anti-VEGF therapy in such injuries, especially as regards the group of patients presenting signs of inflammation, be it because more time had passed from the moment when the injury took place to the treatment of the wound, or because the wound was so extensive, that it became inflamed relatively quickly. As mentioned above, VEGF is the key factor for angiogenesis in numerous pathological eye neovascularisations and is also the cause of numerous patho-

logical inflammation mechanisms, as well as endothelial dysfunctions. Considering the results of our study, in which a significant increase of the vascular endothelial growth factor (VEGF) was recorded in patients with perforating eye injuries, we believe that there is a possibility to improve the conditions of the aforementioned patients and to hasten their sight recovery by applying anti-VEGF therapy, be it by using the already known forms of such therapy, or by discovering new ways for blocking VEGF (e.g. a soluble receptor which would competitively block the free VEGF).

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## VASKULARNI ENDOTHELNI FAKTOR RASTA U OČNIM VODICAMA BOLESNIKA S PERFORATIVNOM OZLJEDOM OKA

### SAŽETAK

S obzirom da je VEGF ključni faktor angiogeneze, a vaskularizacija i vezivna proliferacija odgovorne su za nastanak komplikacija u pacijenata s perforativnom ozljedom, cilj ovog istraživanja bio je utvrditi postoji li povišenje VEGF-a u navedenim stanjima i koliko ovisi o veličini rane, prisutnosti upale, dolaznoj vidnoj oštini, vremenu proteklom od ozljede do obrade rane, te o prisutnosti intrabulbarnog stranog tijela. U ispitivanje je bilo uključeno 20 pacijenata koji su zadobili perforativnu ozljedu oka i njima je za vrijeme operacije obrade rane uzimana očna vodica u kojoj je određivana količina VEGF-a ELISA testom. U očnim vodicama pacijenata sa perforativnom ozljedom i izraženim znakovima upale, ranom većom od 2 mm, te u pacijenata kod kojih je od ozljede do obrade rane prošlo više od 4 sata bila je izmjerena značajno veća količina VEGF-a. Primjena anti-VEGF terapije ima smisla u pacijenata sa perforativnom ozljedom i znakovima upale, ranom većom od 2 mm, te u pacijenata kod kojih je od ozljede do obrade rane prošlo više od 4 sata.